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THERAPEUTIC EFFECTIVENESS OF HUMAN PLATELETS FREEZE-PRESERVED WITH
1974 C R VALERI

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of fresh platelets. The viable platelets were hemostatically effective and were able to reduce the bleeding time in thrombocytopenic patients.

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THERAPEUTIC EFFECTIVENESS OF HUMAN PLATELETS FREEZE-PRESERVED WITH DIMETHYL-SULFOXIDE AT -80 C

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Abstract. DMSO was added to platelet concentrates to a final concentration of 5 or 6%, and the platelet-DMSO mixture was frozen at 2 C per minute in a polyolefin plastic container held in an aluminum container and placed in a -80 C mechanical refrigerator. The platelet concentrates were stored for at least 6 months, and after thawing they were washed by a dilution-centrifugation procedure. The number of these platelets in the circulation 2 hours after transfusion was about 40 to 50% of the number observed after the transfusion of fresh platelets. The viable platelets were hemostatically effective and were able to reduce the bleeding time in thrombocytopenic patients.

Introduction. Our laboratory has developed a simple approach to freeze-preserve human platelets using dimethylsulfoxide (DMSO) as the cryoprotective agent (Valeri et al, 1974). We have evaluated various factors which might be expected to influence the quality of platelets freeze-preserved by any method adopted for largescale use. These include: 1) anticoagulant used for collection; 2) isolation of platelet concentrates from blood; 3) temperature and length of storage before freeze-preservation; 4) type of cryoprotective agent and concentration at the time of freezing; 5) rate of freezing, temperature, and length of frozen storage; 6) washing system; and 7) postthaw storage.

The question of platelet isolation has not yet been resolved. One course is to isolate the platelets from individual units of ACD- or CPD-collected blood and to pool the platelets. Another approach is to use plateletpheresis to collect and isolate several units of platelet concentrates from the blood of a single donor and to return the red blood cells and platelet-poor plasma to the donor.

The platelet preservation method is evaluated by the recovery in vitro of the platelets from the blood, the recovery after the freeze-thaw-wash procedure, the recovery in vivo, and the platelet lifespan. The number of original platelets circulating 2 hours after transfusion and the lifespan of the platelets must be established, as well as the hemostatic effectiveness of the platelets (Handin and Valeri, 1971).

Materials and Methods. We isolated platelet concentrates from 450 ml of citrate-phosphate-dextrose- (CPD) collected blood at room temperature using

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W.S., 8, 48

Anticoagulant	CPD
Method of Isolation	Serial Centrifugation
Final DMSO Concentration	6%
Storage at 22°C Pre-Freeze	2 Hours
Freezing Rate	~2.0C/Min.
Storage Time at -80C	2.4 Months

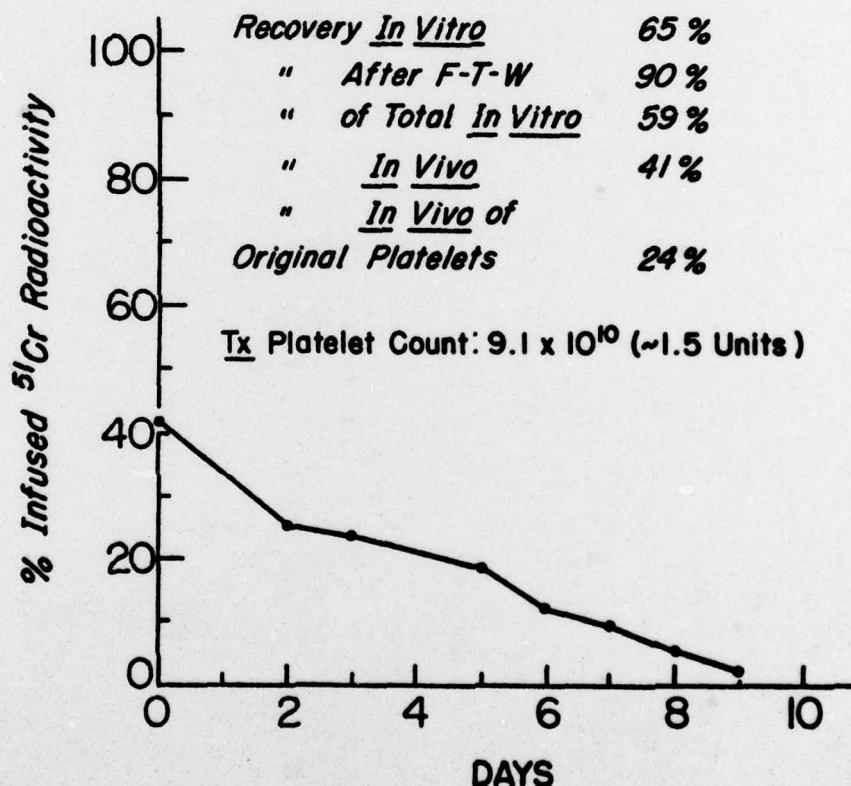


Figure 1. Two units of platelet concentrates were isolated from blood collected from W.S. by plateletpheresis in CPD using serial centrifugation, stored at room temperature for 2 hours, frozen with 6% DMSO at about 2 C per minute in a -80 C mechanical refrigerator, stored at -80 C for 2.4 months, thawed, washed by a single dilution-centrifugation procedure, and stored at room temperature for about 4 hours prior to transfusion. After the autologous transfusions of these platelets, the ^{51}Cr survival, recovery in vitro, recovery in vivo, the lifespan, and the recovery in vivo of the original platelets were measured.

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serial centrifugation as previously described (Valeri et al, 1974). Plateletpheresis is another approach that we used to isolate 2 units of platelet concentrates from blood collected in CPD from a single volunteer using serial centrifugation at room temperature. By plateletpheresis, platelet concentrates were also isolated from blood collected in acid-citrate-dextrose (ACD) using intermittent continuous-flow centrifugation in the Haemonetics Blood Processor 10 in a manner described by Tullis et al (1971) and by Szymanski et al (1973).

Platelet concentrates isolated from 2, 3, or 4 units of blood were pooled, resuspended in 50 ml of plasma, and stored at room temperature for up to 8 hours. To this mixture 50 ml of plasma containing 8%, 10%, or 12% DMSO was added over a 10-15 minute period with agitation at 220 rotations per minute on an Eberbach shaker. The platelet-DMSO mixture was placed in a bioriented polyolefin plastic bag held in an aluminum container and was frozen at about 2 C per minute by storage in a -80 C mechanical refrigerator (Valeri et al, 1974). The platelets were stored for as long as 6 months either at -80 C alone or at -80 C followed by -150 C. The platelets were thawed without agitation, washed by dilution with 100 ml of plasma containing 2% DMSO and 20 ml of ACD, centrifuged to remove all the supernatant, and stored in 30 ml of plasma at room temperature for up to 4 hours before transfusion. It took about 30 minutes to wash 12 units of platelet concentrates.

Platelet circulation was measured by a ^{51}Cr labeling procedure as previously described (Valeri et al, 1974) and by an increase in platelet count following transfusion into thrombocytopenic patients.

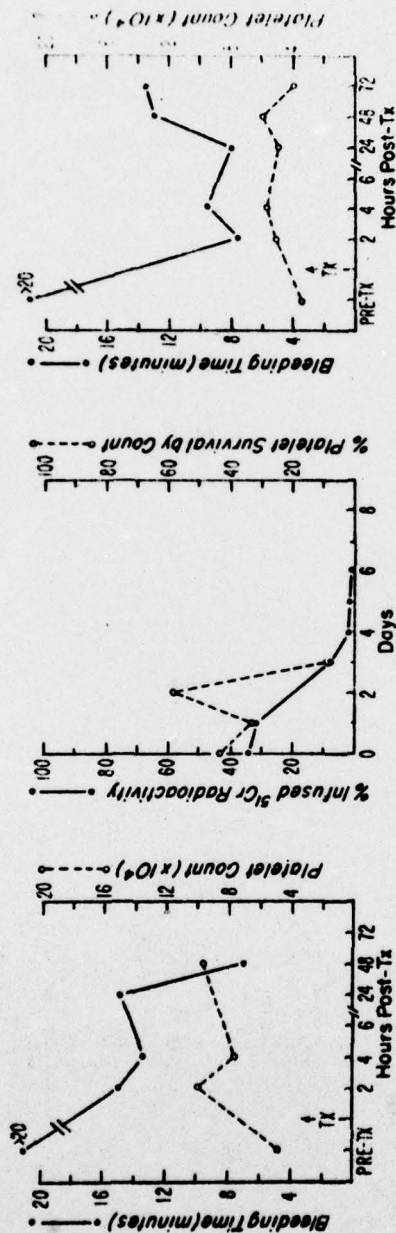
Results. When serial centrifugation was used to isolate the platelet concentrate from CPD blood, the in vitro recovery value was 65%. After storage at room temperature for 24 hours the platelets had excellent circulation: recovery values in vivo of about 50% 2 hours after transfusion, ^{51}Cr T-1/2 values of 4 days, and lifespan values of 8.5 days.

Double units of platelet concentrates isolated from CPD blood by serial centrifugation, frozen with 5 or 6% DMSO at about 2 C per minute and stored at -80 C for about 6 months, had freeze-thaw-wash recovery of about 70%, recovery in vivo of about 35%, ^{51}Cr T-1/2 value of 4 days, and linear lifespan of 8 days (Figure 1). The platelets were able to reduce the prolonged bleeding time and increase the platelet count in patients with thrombocytopenia (Figure 2). Washing reduced the DMSO by at least 90%, and the residual DMSO was about 400 mg per unit. We noted no significant differences whether 4%, 5%, or 6% DMSO was used to freeze the platelets at about 2 C per minute in a -80 C mechanical

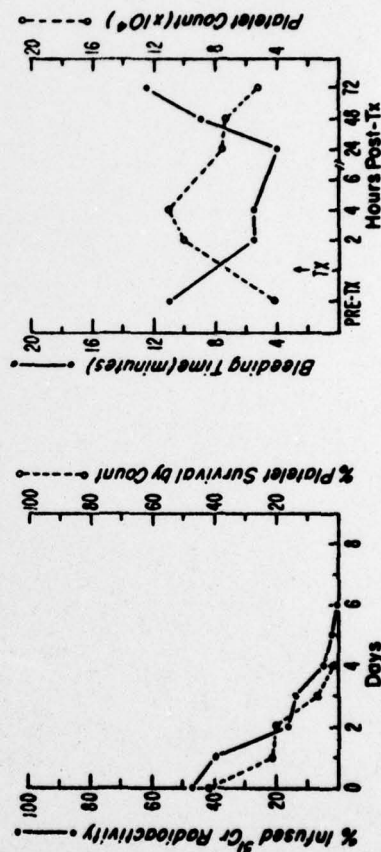
E.B. 67 - Lymphosarcoma

Figure 2.
Therapeutic effectiveness of multiple unit transfusions of platelet concentrates prepared either by liquid storage at 22 C for 24 hours, or by freeze-preservation with 5% DMSO at 2 C per minute and storage at -80 C for up to 30 days before washing. The platelet count, bleeding time, and platelet survival measured by ^{51}Cr labeling and by an increase in platelet count are reported for E.B., a patient with lymphosarcoma and thrombocytopenia.

PLATELETS FROZEN WITH 5% DMSO & STORED AT -80C FOR 30 DAYS
1.5 x 10¹¹ PLATELETS ≈ 2.5 UNITS



PLATELET CONCENTRATES STORED AT +22C FOR 24 HOURS
8.2 x 10¹¹ PLATELETS ≈ 13.5 UNITS



refrigerator (Figures 3A and 3B). Nor were there any significant differences between platelets stored for 6 months at -80 C alone and those stored at a combination of -80 C and -150 C (Figure 4).

When we used intermittent continuous-flow centrifugation in the Haemonetics Blood Processor 10 with the disposable polycarbonate bowl to isolate platelet concentrates from ACD-collected blood, the platelets frequently adhered to the disposable bowl. There were many inconsistencies in the results, and platelets stored for 1 month at -80 C had poor in vivo circulation (Figure 5).

Discussion. In a previous study in our laboratory we found that washing DMSO-preserved platelets after thawing improved the circulation in vivo (Handin and Valeri, 1972). Djerassi and his associates (1966) and Slichter and Harker (1972), on the other hand, do not believe that postthaw washing of DMSO-preserved platelets is necessary. Results of another of our studies revealed no significant differences between platelets freeze-preserved with 5% DMSO at 1 C per minute in a special freezing machine by storage at -150 C for 24 hours and platelets freeze-preserved with 6% DMSO at about 2 C per minute by storage in a -80 C mechanical refrigerator for 24 hours (Valeri, 1974).

In this study plateletpheresis was used to isolate multiple units of platelet concentrates from blood collected in CPD or ACD from a single donor. Serial centrifugation was used to isolate the platelets from CPD blood, and intermittent continuous-flow centrifugation with the Haemonetics Blood Processor 10 was used to isolate platelets from ACD blood. After freeze-preservation with 4%, 5%, or 6% DMSO at -80 C or -150 C for as long as 6 months, the platelets from the CPD blood had recovery values in vitro of about 70%, and circulation in vivo was about 40% that obtained with fresh platelets. Platelets from the ACD blood were freeze-preserved with 4%, 5%, or 6% DMSO at -80 C, and after 1 month of storage the results were unsatisfactory. The recovery values in vitro were lower than those reported by Tullis et al (1971) and by Szymanski et al (1973). These platelets were damaged during the isolation procedure and were not able to tolerate freeze-preservation. Until someone solves the technical problems associated with the isolation procedure using intermittent continuous-flow centrifugation in the polycarbonate disposable bowls, this approach cannot be considered for largescale clinical use.

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Anticoagulant
Method of Isolation
Final DMSO Concentration
Storage at 22°C Pre-Freeze
Freezing Rate
Storage Time at -80°C

CPD
Serial Centrifugation
4%
4.8 Hours
-2.0°C/Min.
4.5 Months

CPD
Serial Centrifugation
5%
4 Hours
-2.0°C/Min.
5.7 Months

CPD
Serial Centrifugation
6%
6.8 Hours
-2.0°C/Min.
4.3 Months

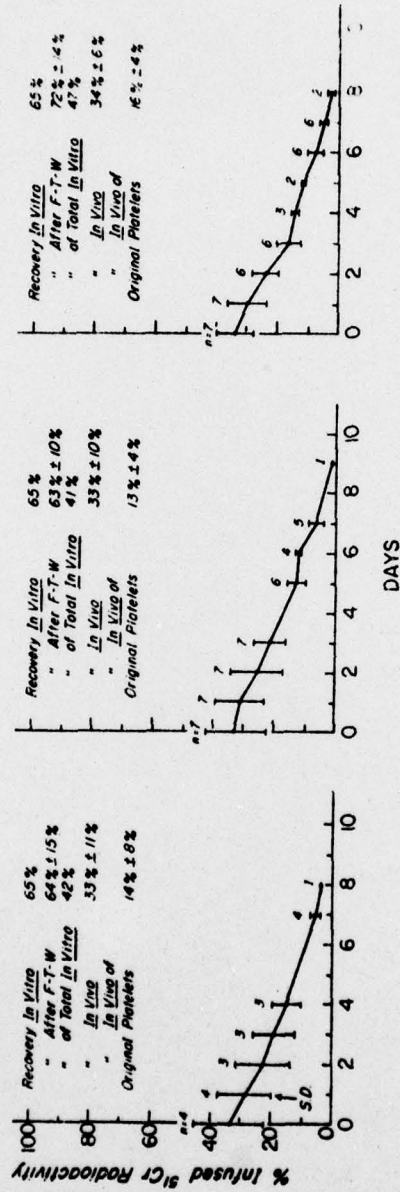


Figure 3A. ^{51}Cr survival of autologous platelets isolated from CPD blood. The platelets were isolated at room temperature by serial centrifugation, and then stored at room temperature for up to 6.8 hours. They were frozen with 4%, 5%, or 6% DMSO at about 2 C per minute by storage in a -80 C mechanical refrigerator. After about 5 months they were thawed, washed, and stored at room temperature for (about 4 hours) prior to transfusion. This figure reports the recovery in vitro and in vivo, the lifespan, and the recovery in vivo of the original platelets.

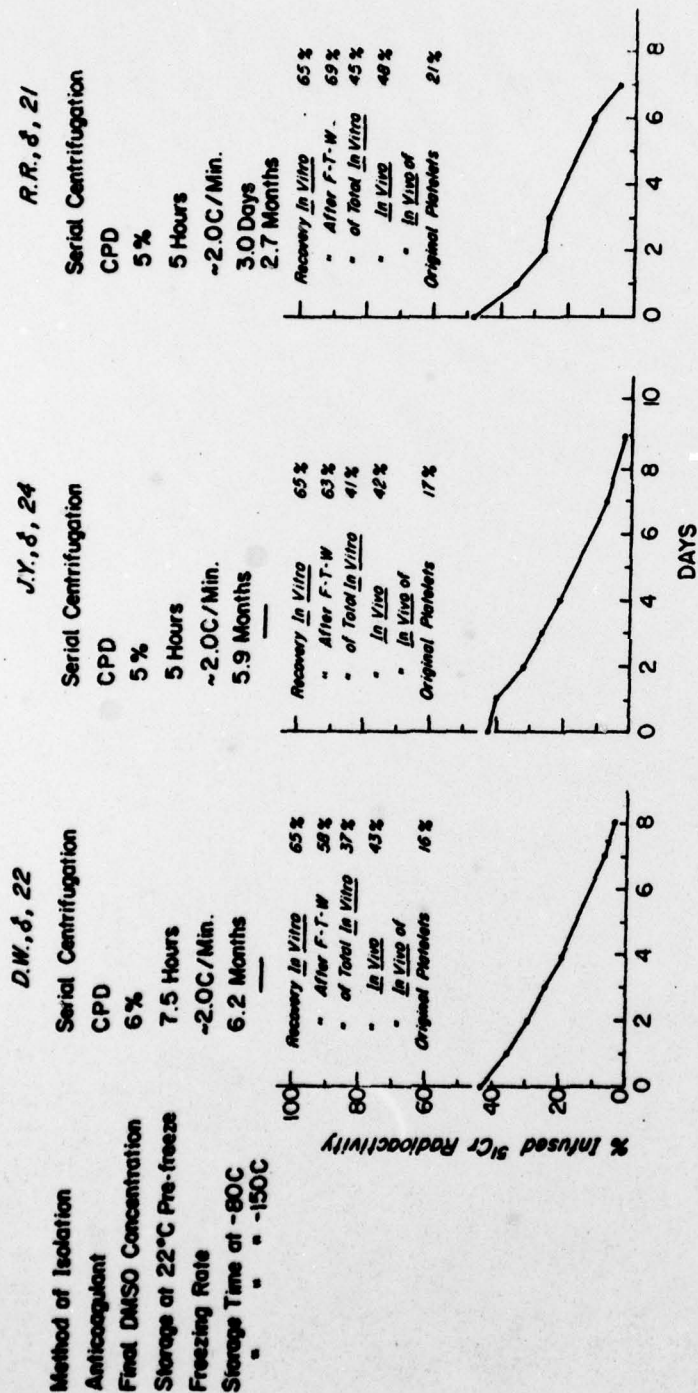


Figure 3B. Platelet concentrates isolated from CPD-collected blood from three volunteers (D.W., J.Y., and R.R.) by serial centrifugation were stored at room temperature for 5 to 7-1/2 hours, frozen at about 2 C per minute with 5 or 6% DMSO, and then stored either at -80 C alone or at -80 C and -150 C for about 6 months. The platelets were thawed at 37 C without agitation, washed by a single dilution-centrifugation procedure, and stored at room temperature for about 4 hours prior to transfusion. After autologous transfusions of these platelets, ⁵¹Cr survival, recovery in vitro, recovery in vivo, the lifespan, and the recovery in vivo of the original platelets were measured.

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Anticoagulant
Method of Isolation
Final DMSO Concentration
Storage at 22°C Pre-Freeze
Freezing Rate
Storage Time at -80°C
" " -150°C

CPD
Serial Centrifugation
5%
4 Hours
-2.0°C/Min.
5.7 Months

CPD
Serial Centrifugation
5%
5.5 Hours
-2.0°C/Min.
2.7 Days
4.1 Months

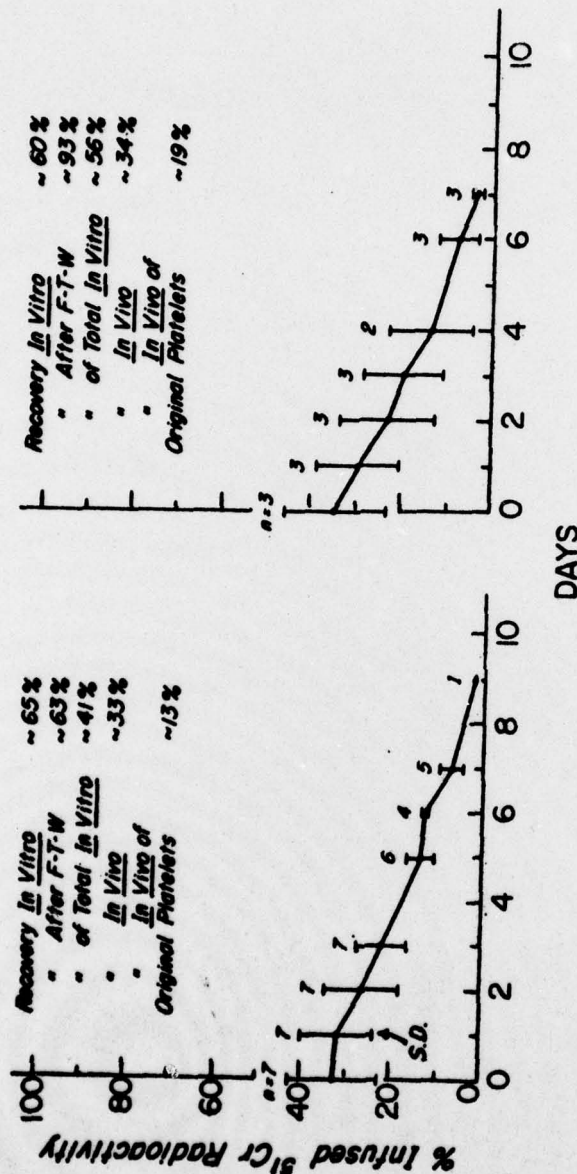


Figure 4. Platelet concentrates were isolated from CPD blood by serial centrifugation, stored at room temperature for 4 to 5.5 hours, frozen with 5% DMSO at about 2 C per minute in a -80 C mechanical refrigerator, stored either at -80 C alone or at -80 C and -150 C for about 6 months, thawed, washed, and stored at room temperature for about 4 hours prior to transfusion. After autologous transfusion of these platelets, recovery in vitro, recovery in vivo, the lifespan, and the recovery in vivo of the original platelets were measured.

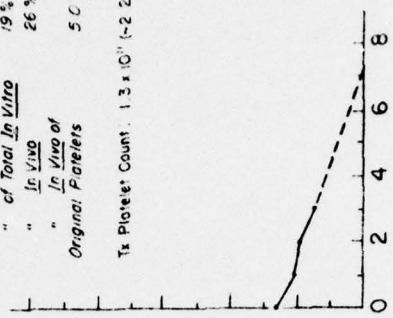
R.H., 8, 38

ACD

- 4 Passes
- 5 %
- 2 Hours
- 2.0°C/Min.
- 26 Days

Recovery In Vitro	19 %
- After F-T-W	98 %
- of Total In Vitro	19 %
- In Vivo	26 %
- In Vivo of Original Platelets	5.0 %

Tx Platelet Count: 1.3×10^{11} (-2.2 Units)



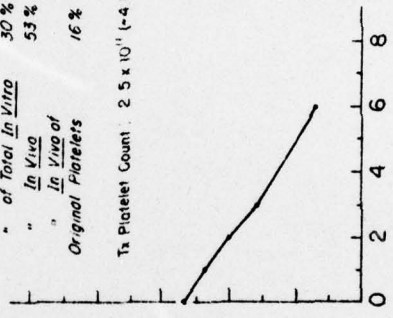
J.F., 8, 38

ACD

- 4 Passes
- 6 %
- 2 Hours
- 2.0°C/Min.
- 1 Day

Recovery In Vitro	32 %
- After F-T-W	94 %
- of Total In Vitro	30 %
- In Vivo	53 %
- In Vivo of Original Platelets	16 %

Tx Platelet Count: 2.5×10^{11} (-4.1 Units)



J.F., 8, 38

ACD

- Anticoagulant
- Haemonetics Processor 10
- Intermittent Cont. Flow
- Final DMSO Concentration
- Storage at 22°C Pre-Freeze
- Freezing Rate
- Storage Time at -80C
- 2 Passes
- 4 %
- 2 Hours
- 2.0°C/Min.
- 14 Days

Recovery In Vitro	25 %
- After F-T-W	81 %
- of Total In Vitro	20 %
- In Vivo	59 %
- In Vivo of Original Platelets	12 %

Tx Platelet Count: 1.2×10^{11} (-1.9 Unit)

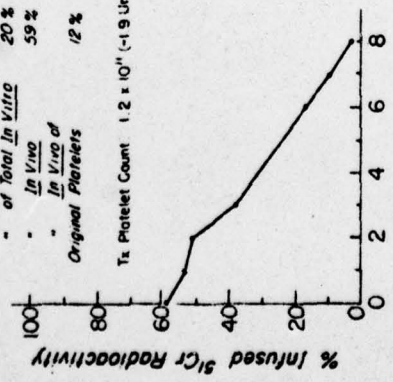


Figure 5. ⁵¹Cr survival of multiple units of autologous platelet concentrates isolated from blood collected by plateletpheresis in ACD. The platelet concentrates were isolated by intermittent continuous-flow centrifugation in the Haemonetics Blood Processor 10 with polycarbonate disposable bowls, and were frozen with 4%, 5%, or 6% DMSO at about 2°C per minute by storage in a -30°C mechanical refrigerator for 1 to 26 days. The thawed platelet concentrates were washed and stored at room temperature for about 4 hours prior to transfusion. This figure shows the recovery in vitro and in vivo, the lifespan, and the recovery in vivo of the lifespan, and the recovery in vivo of the original platelets.